Relationships Between Reserpine Induced Phasic EEG Activity and Single Unit Discharge in the Pontine Reticular Formation and Lateral Geniculate Body Neurons

Attention has recently been focused on so-called monophasic waves recorded in chronic cats during paradoxical sleep (PS) in the pontine reticular formation (PRF) and lateral geniculate body (LGB)[1]. These sharp waves (100–200 μV, 70–100 msec) occur spontaneously, in bursts of 2–8 elements, at a 8/sec frequency, and are time-related to the outbursts of PS, such as eye movements. Similar waves are recorded in chronic cats after a single injection of reserpine (0,5 mg/kg). During 18–20 h the animal is drowsy, and exhibits fast low voltage EEG activity, tonic activity in the nuchal muscles and a permanent discharge of monophasic waves in PRF and LGB. The specific effect of reserpine upon these structures is also observed in acute preparations[2]. Evidence can be obtained that the waves, either during PS or under reserpine, originate in the PRF and then propagate rostrally. For example, in pre-pontine decerebrated cats, PS is still present and the waves can be recorded in the PRF. Moreover, under reserpine LGB-waves are not suppressed by caudal sections of the brainstem (encephale isole or retro-pontine) but disappear after a mid-pontine transection or a bilateral coagulation of a small area in the lateral Tegmentum of the PRF, caudal to this section.

In this study, acute reserpinized cats under Flaxedil were used. The recording started 1 or 2 h after the end of ether anesthesia. Reserpine doses were introduced i.v. Unit activity was recorded extracellularly with 3 M/KCL filled micropipettes and deep EEG with bipolar steel electrodes. 34 neurons, 22 in PRF, 12 in LGB, studied for at least 20 min were selected. In the PRF the 2 electrodes with tips separated by 2 mm were inserted simultaneously with a 60° inclination. Histological controls have shown that the major structure reached was the Nucleus reticularis pontis caudalis. In the LGB, the electrodes were implanted stereotaxically and with reference to the response elicited by flashes of light.

Results. In general, all PRF and LGB cells presenting a clear relationship with the EEG phasic activity showed an obvious increase in firing rate. But the precise relationship between the course of each wave and the corresponding spike discharge rate was studied using specially constructed histograms: for each cell, single waves were superimposed and the number of spikes in corresponding discharges was added every 15 msec between 100 msec before and 100 msec (or more) after the waves.

In the PRF, 3 types of relationship are possible:

(1) Arrest of firing (spontaneously active cells) during the first part of the wave, 10–15 msec after its beginning, followed by an acceleration during the second part: firing starts again 20 msec later, and then progressively increases reaching a maximum at the end of the wave. At this time the rate is about twice that of the background level. During bursts of waves acceleration is abruptly interrupted by the beginning of the next wave (Figure 1, a).

(2) Acceleration of firing during each wave (spontaneously silent cells). Firing occurs only during the wave, starting 15–20 msec after its beginning and reaching a maximum rate 20 msec later, which corresponds to the peak of the wave. The return to zero is progressive, completed about 50 msec later (Figure 1, b).

(3) Continuous acceleration of firing during the bursts of waves without respect to the number of waves. This effect can reach its maximum either immediately, with the first wave of the burst, or more progressively.

In the LGB, all the units selected were spontaneously active and responded to stimulation by flashes of light. Only 1 type of relationship was found. Acceleration of firing starts immediately at the beginning of the wave, and the maximum rate (5–10 times the background level),

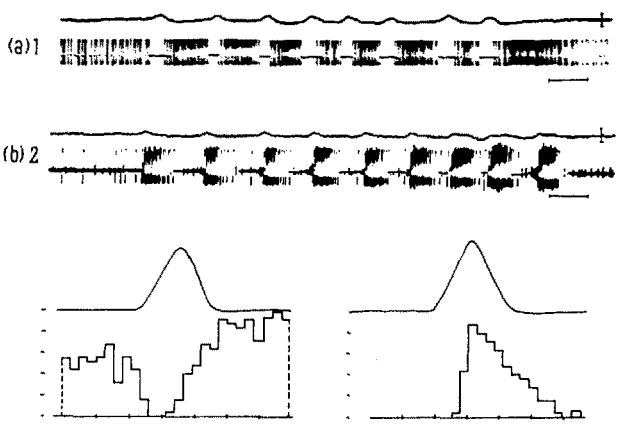

Fig. 1. Pontine reticular formation (PRF) recordings during bursts of reserpine-induced phasic activity. For each pair of records, the upper trace is the deep EEG (bipolar recording), and the lower trace is an extra-cellular record of spike activity. (a) type 1, spontaneously active cell, with a marked slowing of the spike discharge during the first part of the wave, which appears clearly on the left 'histogram' corresponding to the same unit. (b) type 2, spontaneously silent cell, with a burst corresponding to each wave. See corresponding 'histogram' on the right. Calibration: Records: 100 msec and 100 μV. Time constant for unit recordings is 0.001 sec. Histograms: horizontal, each mark represents 50 msec; vertical, each mark represents 10 spikes.

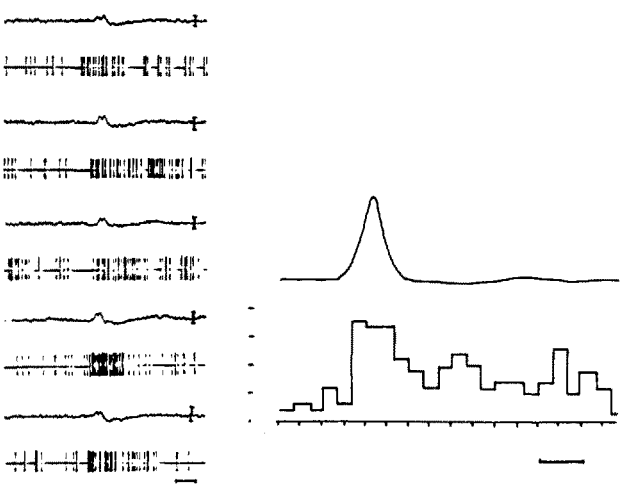

Fig. 2. Extra-cellular recording of a LGB unit during single phasic waves. Records corresponding to 5 waves are superimposed (left). On the right, the histogram is from the same cell. Calibration is the same as in Figure 1.

[1] M. Jouvet and F. Michel, C. r. Séanc. Soc. Biol. *153*, 422 (1959); T. N. Mikiten, P. H. Niebyl and L. D. Hendley, Fedn Proc. Fedn Am. Socs exp. Biol. 20, 237 (1961).

[2] M. Jouvet, M. Jeannerod and F. Delorme, C. r. Séanc. Soc. Biol. *159*, 1599 (1965).

reached rather abruptly, coincides with the peak of the waves. The decrease is then progressive through several hundreds of msec (Figure 2). During repetitive stimulation by flashes before the injection of reserpine, all the units responded by short bursts of 1–4 spikes. Under reserpine, the same response was found but the discharges corresponding to the phasic waves were superimposed. The EEG evoked potentials occuring during this increase of firing were consistently reduced in size. This effect is clearly observed for a frequency of flashes around 15/sec, but seems to disappear if higher than 25/sec.

Comments. (1) Phasic EEG waves observed in PRF and LGB after a single injection of reserpine do not reflect in a simple and consistent manner the rate of the spike activity of the neurons. We may then hypothetize that they are related to post-synaptic events occuring in those groups of cells. The rate changes which occur during each phase of the reserpine-waves could then provide suggestions as to the excitatory and inhibitory processes involved in the generation of these waves. In the PRF, most neurons apparently receive inhibitory input during the first part of the wave, and then present a rate acceleration which can be interpreted as due to a post-inhibitory rebound, or a superimposed excitatory effect (type 1). The other cells (type 2 and 3) are suggestive of a predominantly excitatory input. Note however, for type 2 cells, that the possibility of some early inhibition cannot be rejected since they are spontaneously silent.

In the LGB, all the neurons recorded accelerated, suggesting an extensively excitatory process. The LGB-wave could thus reflect the depolarization of this group of cells by synchronous EPSPs. 2 discharge generating processes are thus represented by the same EEG pattern recorded in 2 different areas.

(2) These experiments provide some evidence as to the effect of reserpine at the neuronal level-other evidence has indicated that the site of action of reserpine in the brainstem should be located in the PRF[2]. Pontine neurons influenced by reserpine project to the LGB, 'non-specific' convergence which is confirmative of the demonstration of other authors[3]. But although the visual input to the geniculate neurons, responsible of a very short depolarization, gives rise to 1 or few spikes, the non-specific one causes a longer depolarization and gives rise to repetitive spikes. This difference of response could reflect a difference of location of the corresponding synapses on the cell membrane[4].

(3) The similarity of the phasic EEG patterns in reserpine preparations and during PS raises the question of whether they obey similar mechanisms at the neuronal level. Several workers have observed during PS phasic increases of cell discharges corresponding to the bursts of eye movements (which are known to be closely related in time with the phasic waves), in many structures including LGB[5], which is in agreement with our findings in this structure. But, so far, no study has been performed in the PRF during PS so that we might compare with our results[6].

Résumé. Des ondes rapides sont enregistrées dans la formation réticulée pontique (FRP) et le corps géniculé latéral (CGL) chez des chats sous Reserpine. Au moment des ondes rapides, l'activité des neurones de la FRP est généralement inhibée, parfois augmentée. Elle est toujours augmentée dans le CGL. Un rapprochement est tenté avec des phénomènes semblables observés pendant le sommeil paradoxal.

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[3] G. B. ARDEN and U. SODERBERG, in *Sensory Communication* (Ed. W. A. ROSENBLITH; M.I.T. Press, Cambridge 1961) p. 521.
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[6] Supported by a grant from N.I.H. (No. 5264). Thanks are due to Prof. J. P. SEGUNDO, Dept. of Anatomy, U.C.L.A., for support and criticism.
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Nichtenzymatisch bedingte TNBT-Formazanablagerungen in Gewebsstrukturen

Tetra-Nitro-Blue-Tetrazolium (TNBT) gilt gegenwärtig in der Fermenthistochemie als zuverlässigster Reduktionsindikator zum histochemischen Nachweis oxydativer Enzyme. Eigene lichtmikroskopische Untersuchungen haben ergeben, dass sich in Gewebsschnitten nach kurzzeitiger Inkubation in einer TNBT-Lösung und anschliessender Reduktion mit Ascorbinsäure oder Hydrochinon nichtenzymatisch gebildetes TNBT-Formazan nachweisen lässt. Gleichartige Ergebnisse sind inzwischen auch von LOJDA[1], BROOKE und ENGEL[2] sowie FAHIMI und KARNOVSKY[3] mitgeteilt worden. Im Gegensatz zu den genannten Autoren haben wir ausserdem elektronenmikroskopische Untersuchungen durchgeführt, über die im folgenden berichtet werden soll. Die dabei erzielten Ergebnisse werfen die Frage auf, ob bei Verwendung von TNBT als Reduktionsindikator zum histochemischen Nachweis oxydativer Enzyme mit einer unspezifischen TNBT-Formazanbildung zu rechnen ist.

Kryostatschnitte nativer und glutaraldehydfixierter Rattenherzmuskulatur wurden 5–20 min in einer gepufferten TNBT-Lösung inkubiert und anschliessend mit Hydrochinon bzw. Ascorbinsäure zum TNBT-Formazan reduziert. Ein Teil der Schitte wurde mit OsO_4 nachfixiert. Die Einbettung der Kryostatschnitte für elektronenmikroskopische Untersuchungen erfolgte in Vestopal W, Araldit und Durcupan-Fluka. Zur Klärung der Frage, ob

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